PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

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PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/JP2004/014696	29.09.2004	30.09.2003

International Patent Classification (IPC) or both national classification and IPC
C12N9/04, C12N15/82, C12P7/22, A01H5/00

Applicant
SUNTORY LIMITED

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:

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WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/JP2004/014696

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	10,14-20
	No:	Claims	1-9,11-13,21
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-21
Industrial applicability (IA)	Yes:	Claims	1-21
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1 Reference is made to the following documents:

D1: XIA Z-Q ET AL: "Dirigent-mediated podophyllotoxin biosynthesis in Linum flavum and Podophyllum peltatum" PHYTOCHEMISTRY, vol. 55, no. 6, November 2000, pages 537-549, XP004291678

D2: GANG D R ET AL: "REGIOCHEMICAL CONTROL OF MONOLIGNOL RADICAL COUPLING: A NEW PARADIGM FOR LIGNIN AND LIGNAN BIOSYNTHESIS" CHEMISTRY AND BIOLOGY, CURRENT BIOLOGY, vol. 6, no. 3, March 1999 pages 143-151, XP000995932

D3: OVERKAMP STEFAN ET AL: "Cloning and characterization of eight cytochrome P450 cDNAs from chickpea (Cicer arietinum L.) cell suspension cultures" PLANT SCIENCE, vol. 155, no. 1, June 2000, pages 101-108, XP002314013

D4: DATABASE EMBL 12 December 2001, "Arabidopsis thaliana cytochrome P450-like protein (F6G17.20) mRNA, complete cds." XP002314014 retrieved from EBI accession no. EM_PRO:AY065192 Database accession no. AY065192

D5: DATABASE EMBL 5 June 2002, "Arabidopsis thaliana putative cytochrome P450 protein (At3g28740) mRNA, complete cds." XP002314015 retrieved from EBI accession no. EM_PRO:AY113869 Database accession no. AY113869

D6: DATABASE EMBL 27 August 2001, "Arabidopsis thaliana putative cytochrome P450 protein (At3g28740) mRNA, complete cds." XP002314016 retrieved from EBI accession no. EM_PRO:AY050849 Database accession no. AY050849

D7: DATABASE EMBL 14 June 2002, "Arabidopsis thaliana clone 253698 mRNA, complete sequence." XP002314017 retrieved from EBI accession no. EM_PRO:AY086486 Database accession no. AY086486

D8: JIAO YING ET AL: "Furanofuran lignan metabolism as a function of seed maturation in Sesamum indicum: Methylenedioxy bridge formation" PHYTOCHEMISTRY, vol. 49, no. 2, September 1998, pages 387-394, XP004290112

D9: IKEZAWA NOBUHIRO ET AL: "Molecular cloning and characterization of

CYP719, a methylenedioxy bridge-forming enzyme that belongs to a novel P450 family, from cultured Coptis japonica cells." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 278, no. 40, 3 October 2003, pages 38557-38565, XP002313984

2 The present application does not meet the criteria of Article 33(1) PCT, because the subject matter of **claims 1 to 9, 11, 12 and 21** is not new in the sense of Article 33(2) PCT.

Documents D4 to D7 disclose cDNAs encoding proteins, which are at least 50% identical to the proteins shown in SEQ ID NO: 1, 64 or 78 (cf. the whole documents). These proteins are expected to have the same enzymatic activity as the protein described in the present application. The nucleic acids encoding these proteins hybridize under stringent conditions to the nucleic acids disclosed in the application at issue.

Document D3 is prejudicial to the novelty of the subject matter of **claims 1 to 9, 11 to 13 and 21**. It describes cDNAs encoding proteins, which are more than 50% identical to the proteins shown in SEQ ID NO: 1, 64 or 78 (cf. D3, figure 2). These cytochrome P450 proteins are expected to have the same enzymatic activity as the protein described in the present application. The nucleic acids encoding these proteins hybridize to the nucleic acids disclosed in the application at issue. Document D3 also describes the expression of these proteins in yeast (cf. D3, page 103).

The subject matter of present **claims 1, 7, 8, 11 to 13 and 21** lacks novelty having regard of the disclosure of documents D1 and D2 (Article 33(2) PCT). These documents describe nucleic acids encoding enzymes, which are involved in the synthesis of pinoresinol, the direct precursor of piperitol, and are therefore considered to represent genes encoding proteins catalysing the biosynthesis of piperitol (cf. D1, figures 7, 8; D2, figure 2).

The origin of a nucleic acid, e. g. a nucleotide "derived from sesame", does not allow to distinguish it from a nucleic acid, which has a structure falling within the scope of the claims, but which has been derived from a different organism.

The subject matter of **claims 8 and 9** is not new in view of the disclosure of document D8, which describes a purified microsomal preparation containing the enzyme catalysing the biosynthesis of piperitol (cf. D8, page 393). This protein cannot be distinguished from the protein described in the present application.

3 The subject matter of present **claim 3, part (a)**, relates to a gene encoding a

protein characterized by the sequence shown in SEQ ID NO: 1, 64 or 78.
Document D8, which is considered to represent the closest prior art, describes the partial purification and characterization of an enzyme involved in the formation of piperitol (cf. D8, page 393).
The problem to be solved the present application is considered to reside in the provision of a gene encoding said protein, which would allow *inter alia* the modification of piperitol synthesis *in planta*.
Claim 3, part (a), provides a gene encoding a protein characterized by the sequence shown in SEQ ID NO: 1, 64 or 78, thereby solving the problem. The encoded enzyme catalyses the formation of a methylene dioxybridge in the synthesis of piperitol.
Document D8 describes the purification of a microsomal fraction containing the enzymatic activity responsible for the formation of piperitol. This document characterizes the enzyme as being dependent on NADPH and oxygen, and as belonging to the group of cytochrome P450 enzymes (cf. D8, pages 390 to 392).
Starting from this knowledge, the skilled person would have obtained the protein as well as the gene encoding said protein in a fairly straightforward manner using routine technologies that are well established in the art. Apparently, in the cloning of the enzyme no unexpected difficulties were encountered, which would have required inventive activity to overcome. The feasibility to clone P450 enzymes involved in the formation of methylene dioxybridges has been shown in document D9, which has been available online on the 5th of May 2003.
Since the enzyme does not show any surprising technical effects compared to the enzyme described the prior art (D8), an inventive step cannot be acknowledged.
The same reasoning applies, mutatis mutandis, to the subject matter of the **claims 10 and 14 to 20**, which therefore are also considered not inventive.

4 In **claims 12, 16 and 18**, it should be made clear that the term "transformant" does not extend to human beings.

5 The attention of the applicant is drawn to the fact that the application may not be amended in such a way that it contains subject-matter which extends beyond the content of the application as filed (Article 34(2)(b) PCT).